[Communicative & Integrative Biology 1:2, 125-127; October/November/December 2008]; ©2008 Landes Bioscience

Article Addendum

A clash of stressors and LTM formation

Pascaline de Caigny and Ken Lukowiak*

Hotchkiss Brain Institute; Faculty of Medicine; University of Calgary; Calgary, Alberta Canada

Key words: stress effects on memory, crowding, long-term memory, snail, operant conditioning

Stress alters long-term memory formation sometimes enhancing its formation whilst at other times blocking it. It is unclear what the causal mechanisms are that allow stress to either enhance or suppress memory. We have made use of a relatively simple invertebrate model system to attempt to explore the causal mechanisms of how stress alters memory. Here we explore the consequences of presenting to the organism two different ecologically relevant stressors: detection of a predator and crowding. We find that the suppressive effect on memory formation elicited by crowding is more powerful than is the enhancing effect on predator-detection. That is, when the two stressors are experienced by the snail, long-term memory formation is suppressed.

It is well known that stress, either physical or psychological, alters memory formation.[1,2] In some cases enhancing it whilst in other circumstances suppressing it. This has even been 'formalized' as the Yerkes-Dotson law (reviewed in ref. 2) derived from experiments on rodents in the early 1900's. However, the neural and molecular mechanisms as to how stress modulates memory is not at all clear. One reason for this is the complexity of both the mammalian brain and the behavioural tasks used. In an effort to overcome these constraints we make use of an invertebrate model system, the pond snail *Lymnaea stagnalis*, where we have the opportunity to more easily unravel the neural and molecular mechanisms that underlie memory formation.[3] We have this opportunity because: (1) The behaviour we analyze, aerial respiration, is a simple, easily observable, tractable behaviour that can be operantly conditioned;[3] (2) This behaviour is driven by a 3-neuron central pattern generator (CPG) whose sufficiency and necessity has been experimentally determined;[4,5] and (3) A single, identified neuron, RPeD1 a member of the aforementioned CPG, is a necessary site for long-term memory formation (LTM) following operant conditioning of aerial respiration.[6]

We recently demonstrated that LTM formation is modulated by a number of specific stressors[7] some which are ecologically relevant. For example, exposure of the snail to the scent of a sympatric predator (e.g., crayfish) causes a significant enhancement of LTM formation;[8] whilst subjecting snails to another environmental stressor, crowding, immediately before associative learning blocks LTM formation.[9] If snails are exposed to a predator released karimore (a chemical secreted by a non-conspecific organism that when detected by an organism of a different species, evokes a response in that species that adaptively favors it) they activate various anti-predator behavioural responses. One of these anti-predator responses is an enhanced ability to form LTM. On the other hand, snails subjected to a training procedure that normally results in LTM if they are placed into crowded conditions for 1h prior to training do not form LTM.[9] These findings prompted us to ask what the outcome of memory formation would be following operant conditioning of aerial respiration if we exposed snails to crowding and then to the scent of the predator. Would we see enhanced LTM or no LTM?

In the data presented in Figure 1 we trained snails as described in detail in our recent publications.[8,9] Briefly, snails received a tactile stimulus to the pneumostome area (the respiratory orifice) during the 0.5 h training session (TS1) as they attempted to open it to perform aerial respiration. We then tested for memory 24 h later in a similar 0.5 h session. Memory is operationally defined as a significant decrease in the number of attempted pneumostome openings in the memory test (MT) compared to TS1 using a paired t-test. Three separate cohorts of naive snails were used: (1) Snails maintained under normal conditions (2 snails/100 ml) and trained in hypoxic pond water (PW); (2) Snails maintained under normal conditions (2/100 mL) but trained in crayfish effluent hypoxic pond water (CE); and (3) Snails maintained in crowded conditions (20/100 ml) for 1 h just prior to training in hypoxic pond water CE.

As can be seen (Fig. 1A) training snails with a single 0.5 h training session in PW does not result in LTM when we tested for memory 24 h later. That is, the number of attempted pneumostome openings in MT was not significantly different than in TS1 ($p = 0.487$). However, if we trained another cohort of naive snails for 0.5 h in CE LTM was observed (Fig. 1B). In this cohort the number of attempted pneumostome openings in MT was significantly less than the number in TS1 ($p < 0.01$). Thus, as we have previously shown the detection of a sympatric predator within 30 min prior to or during operant conditioning training causes an enhancement of the ability of the snails to form LTM. We then asked using a third cohort of snails whether predator detection would still cause an enhancement of LTM formation if training in CE was preceded by crowding. Crowding for a period as short of 1 h immediately before training

*Correspondence to: Ken Lukowiak; Hotchkiss Brain Institute; Faculty of Medicine; University of Calgary; Calgary, Alberta T2N 4N1 Canada; Email: lukowiak@ucalgary.ca

Submitted: 08/22/08; Accepted: 08/25/08

Previously published online as a *Communicative & Integrative Biology* E-publication: http://www.landesbioscience.com/journals/cib/article/6858

Addendum to: de Caigny P, Lukowiak K. Crowding, an environmental stressor, blocks long-term memory formation in Lymnaea. J Exp Biol 2008; 211:2678–88; PMID: 18689421; DOI: 10.1242/jeb.020347.

blocks LTM formation. We found here (Fig. 1C) that crowding for 1 h just prior to training in CE blocked LTM formation. That is, the number of attempted pneumostome openings in MT was not significantly different than in TS1 ($p = 0.532$).

While this is certainly not an exhaustive study of all the possible temporal combinations of CE exposure and crowding; the sequence used here is an adequate test of whether exposure to CE can supplant the blocking effect that crowding has on the establishment of LTM. It is well known that stress can either enhance or suppress memory formation.[1] Typically however different stressors are used on different groups of subjects, employing different tasks. Here is one of the first examples we know of asking which of two possible outcomes, LTM or no LTM, will result when an organism is exposed to two different stressors, whose outcome on LTM formation is either enhanced (exposure to predator scent) or suppressed (crowding).

Our initial expectation was that ability of CE to enhance LTM formation would overcome the suppressive effects elicited by crowding on LTM formation. Our reasoning was that the enhanced memory seen following exposure of snails to the scent of a predator was an adaptive, inducible anti-predator response[10] and that inducible anti-predator behavioural responses would always 'win out' over other 'less' important modifications to behaviour. However, as shown here that was not the case. Crowding specifically targets the genomic activity necessary for LTM formation as a shorter form of memory, intermediate-term memory (ITM) while dependent on new protein synthesis is not dependent on altered gene-activity.[11,12] We also have preliminary data that the effects of CE on LTM are dependent on altered gene activity in RPeD1. That is, CE induces changes in genomic activity in RPeD1 that are necessary for the formation of LTM. Previously, it has been shown that RPeD1 is a necessary site for LTM formation,[6] reconsolidation,[13] extinction,[14] and forgetting.[15] Thus, it is quite likely that crowding interferes with CE-induced required altered gene activity necessary for LTM formation. Thus, crowding's effects on altered gene activity are such that CE is no longer able to induce the genomic activity necessary to cause LTM memory enhancement. Why would this happen? It may be that under crowded conditions it is not necessary to expend the 'energy' necessary to induce altered gene activity because of 'herd-protection'. That is, the likelihood of an individual snail being preyed upon is decreased if there are many other snails around. Future experiments will determine if crowding after training in CE will also inhibit the formation of LTM. Based on the data obtained here we predict that it will.

What are the possible consequences of these findings? One possibility is that these data might serve as a model for developing strategies to alleviate psychiatric problems arising, for example, from war-related atrocities leading to post-traumatic stress syndrome (PTSD). PTSD has been defined[16,17] by a specific pattern of core symptoms including alterations in memory formation leading to memories so enhanced that the patients often re-experience the traumatic event and exhibit hyperarousal symptoms. We have shown that predator-detection leads to hyperarousal in snails. Their memory of events at the time of exposure to the predator are better remembered. These data are somewhat analogous to the symptoms experienced by humans following traumatic experiences (e.g., War atrocities, encounters with predators such as wild animals, etc.,). Here we show that it is possible to block the enhancement of LTM by use of another environmental stressor. Whether such a procedure could work in these remains to be seen, but our data do indicate that it is possible to mitigate the effects induced by a traumatic experience, such as detecting a predator.

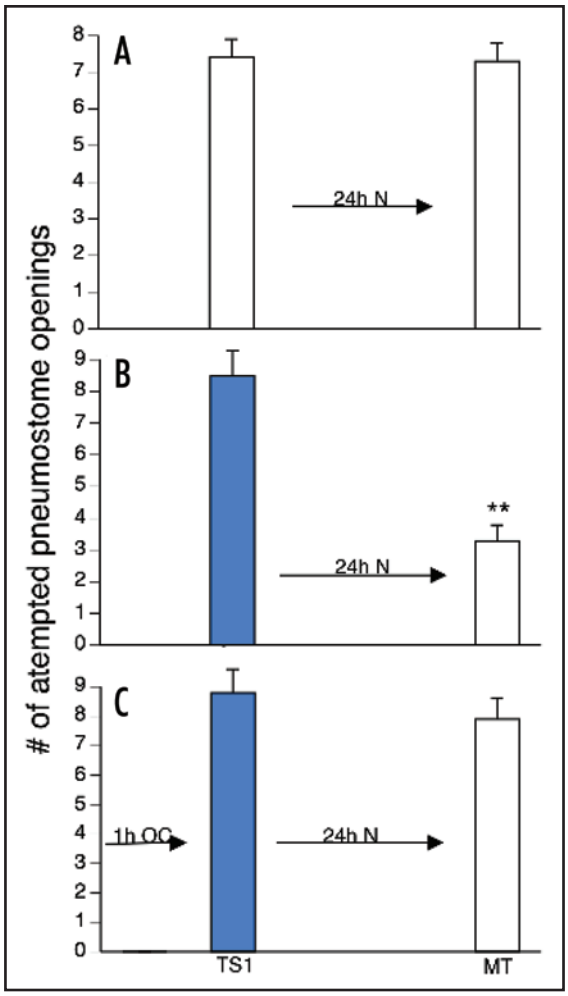

Figure 1. Crowding and exposure to the scent of a predator on LTM formation. (A) In a cohort of naive snails (n = 18) a 0.5 h training session (TS1) does not result in LTM. (B) However when another naive cohort of snails (n = 17) is trained in crayfish effluent (CE), LTM is formed. (C) When snails (n = 18) are overcrowded for 1 h before the 0.5 h TS in CE, LTM does not form.

References

1. Kim J, Diamond D. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002; 3:453-62.
2. Shors T. Stressful experience and learning across the lifespan. Annu Rev Psychol 2006; 57:55-85.
3. Lukowiak K, Ringseis E, Spencer G, Wildering W, Syed N. Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. J Exp Biol 1996; 199:683-91.
4. Syed N, Bulloch AGM, Lukowiak K. In vitro reconstruction of the respiratory central pattern generator of the mollusk Lymnaea. Science 1990; 250:282-5.
5. Syed N, Ridgway R, Lukowiak K, Bulloch AGM. Transplantation and functional integration of an identified respiratory interneuron in *Lymnaea stagnalis*. Neuron 1992; 8:767-74.
6. Scheibenstock A, Krygier D, Haque Z, Syed N, Lukowiak K. The soma of RPeD1 must be present for LTM formation of associative learning in Lymnaea. J Neurophysiol 2002; 88:1584-91.
7. Lukowiak K, Martens K, Rosenegger D, Browning K, de Caigny P, Orr M. The perception of stress alters adaptive behaviours in *Lymnaea stagnalis*. J Exp Biol 2008; 211:1748-56.
8. Orr M, Lukowiak K. Electrophysiological and behavioral evidence demonstrating that predator detection alters adaptive behaviors in the snail Lymnaea. J Neurosci 2008; 28:2726-34.

9. de Caigny P, Lukowiak K. Crowding, an environmental stressor, blocks long-term memory formation in Lymnaea. J Exp Biol 2008; 211:2678-88.
10. Orr El-Bekai M, Lui M, Watson K, Lukowiak K. Predator detection in *Lymnaea stagnalis*. J Exp Biol 2007; 210:4150-8.
11. Sangha S, Scheibenstock A, McComb C, Lukowiak K. Intermediate and long-term memories of associative learning are differentially affected by transcription vs. translation blockers in Lymnaea. J Exp Biol 2003; 206:1605-13.
12. Lukowiak K, Adatia A, Krygier D, Syed N. Operant conditioning in Lymnaea: Evidence for intermediate and long-term memory. Learning & Memory 2000; 7:140-50.
13. Sangha S, Scheibenstock A, Lukowiak K. Reconsolidation of a long-term memory in Lymnaea requires new protein and RNA synthesis and the soma of RPeD1. J Neuroscience 2003; 23:8034-40.
14. Sangha S, Scheibenstock A, Morrow R, Lukowiak K. Extinction requires new RNA and protein synthesis and the soma of the cell RPeD1 in *Lymnaea stagnalis*. J Neuroscience 2003; 23:9842-51.
15. Sangha Scheibenstock A, Martens K, Varshney N, Cooke R and Lukowiak K. Impairing forgetting by preventing new learning and memory. Behav Neurosci 2005; 119:787-96.
16. Elbert T, Schauer M. Burnt into memory. Nature 2002; 419:883.
17. Yehuda R, LeDoux J. Response variation following trauma: A translational neuroscience approach to understanding PTSD. Neuron 2007; 56:19-28.